

Molecular Diagnosis of Multicentric Canine Lymphoma in a Boxer – A Case Report

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Abstract

A 12-year-old cachectic male Boxer weighing around 18.2 kg was presented to the Department of Veterinary Medicine, Veterinary Clinical Complex, RIVER with a history of inappetence, weakness, recumbency, polyuria and polydipsia for the past month. Physical examination revealed multifocal moist erosive plaques on the limbs, icteric conjunctival and buccal mucosae, and generalised lymphadenopathy. Lymph node aspirate was collected in PBS and sent for PARR (PCR for Antigen Receptor Rearrangement assay), which revealed the clonality of B and T-cell receptors, confirming the case as lymphoma. Owing to the senility of the patient and correlated poor prognosis, the owner objected to commence chemotherapy. Therefore, the pet was treated symptomatically. This paper emphasises the applicability of the PARR assay, a sensitive tool to differentiate between types of lymphoma and the associated outcome of the case.

Keywords: Multicentric Canine Lymphoma, Molecular diagnosis, PARR Assay

Introduction

Lymphomas are tumours of hematopoietic origin, defined as a proliferation of malignant lymphoid cells affecting the lymph nodes or solid visceral organs, such

as the liver or spleen (Harvey,1990). They encompass a heterogeneous group of several cancer subtypes arising from lymphoid cells (Vail, 2017) Anatomical, histological and immunophenotypic characteristics form the basis of the classification. Multicentric canine lymphomas are the most frequently occurring manifestation, accounting for 84% of canine lymphomas. The present paper describes the application of PARR assay in the accurate diagnosis of multicentric lymphoma stage IV substage b (WHO, 1980).

Case History and Observation

A twelve-year-old male Boxer weighing 18.2 kg (Fig.1) was presented to the Small Animal Medicine Unit, VCC, RIVER, with a history of inappetence, weakness, recumbency, dark-coloured paradoxical diarrhoea, polyuria and polydipsia. Upon physical examination, the animal was emaciated with generalised skin laxity, multifocal moist erosive plaques on the limbs, icteric conjunctival and buccal mucosae and generalised lymphadenopathy. Skin scraping were negative for mites. Tachypnoea (rapid shallow respiration) 46 breaths/min, tachycardia 151 bpm and delayed CRT lasting more than 2 seconds were observed on clinical examination. Blood samples were collected for routine haematology and serum biochemistry (LFT&KFT). Haematological examination revealed leucocytosis (TLC - 53.3×10^3 cells/ μ L) with severe anemia (TEC - 1.83×10^6 cells/ μ L, Hb - 4.1 g/dL, PCV 13.1%) and mild

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thrombocytopenia (1.1×10^5 cells/ μ L). Serum biochemistry revealed hyperbilirubinemia (3.07 mg/dL), hyperglobulinemia (3.66g/dL) and azotemia (BUN 166mg/dL, creatinine 2.36mg/dL). Electrocardiographic findings indicated an absence of P wave with a low-voltage QRS complex indicative of atrial fibrillation and mild myocardial ischaemia (Fig.2). Thoracic (Fig.3) and abdominal radiographs (Fig.4) revealed miliary pulmonary infiltration, a fluid shadow of the cranial lobe of the lung and hepatomegaly, fluid shadow within the peritoneal cavity (ascites) and bowels obstructed with faeces respectively. Ultrasonographic findings included fibrinous pericarditis, pericardial effusion, intestinal loops with increased mucosal echogenicity and splenomegaly (splenic body thickness: 38.1 mm) (Fig.5).

Lymph node aspirates were collected in PBS for PARR (Polymerase Chain Reaction for Antigen Receptor Rearrangement) assay and sent to the Project Director, Translational Research platform Veterinary Biologicals, TANUVAS, Chennai 51. Lymph node biopsy was taken twice but was inconclusive

Treatment and discussion

Lymph node biopsy is considered the gold standard diagnostic test for lymphoma; however, in the present case, fine needle aspiration cytology was inconclusive, only detecting neutrophils and fibrin, indicative of lymphadenitis. Therefore, molecular diagnosis by PARR assay was considered, which confirmed the clonal expansion in B-cell receptor (BCR) and T cell receptor – Gamma (TCR- γ) (Fig.6). Based on clinical signs, ultrasonographic findings and clonality by PARR assay the case was diagnosed as Multicentric Canine Lymphoma Stage-IV (involvement of multiple lymph nodes, liver and spleen), substage-b i.e poor health status with clinical signs of disease (Harvey,1990). The advanced clinical stage

of the condition and senility factored a poor prognosis. Therefore, the owner objected to the commencement of chemotherapy. Hence, treatment was primarily palliative, and the animal succumbed 2 weeks following the day of the initial presentation.

Lymphomas remain etiologically elusive, and many genetic, infectious, immunological and environmental factors have been postulated as predisposing. Older dogs, 6-12 years of age (Mansoor Lakooraj *et al.* 2018) and certain breeds, such as Boxers, are found to have a propensity towards developing lymphomas (Vail *et al.* 2013). Canine lymphomas are generally diagnosed by microscopic evaluation of fine needle aspirate (FNA) followed by histopathology and immunohistochemistry of affected lymph nodes (Claire Cannon,2018). As diagnosis of lymphoma based on cytologic and histologic features is not always possible and at times inconclusive, a need for an advanced molecular technique may be fundamental to obtain a confirmatory diagnosis and further tumor characterization (Rocha *et al.* 2025). PCR for antigen receptor rearrangement (PARR) is a molecular assay targeting specific DNA sequences of lymphocytes Ig gene of B cells and T cell receptor (TCR) genes as a mode of determining the monoclonality and neoplastic transformation of the sampled lymphocyte population that can aid in confirmation. Uncontrolled monoclonal expansion of lymphocytes tends to be neoplastic rather than reactive (Swerdlow, 2003). The appearance of a single-sized dominant amplicon in the PCR-based approach confirms the clonality. Hence, the assay is termed PCR for antigen receptor rearrangement (PARR). The PARR method enables the detection of even one neoplastic cell in 100 cells and is thus helpful in identifying neoplastic lymphocytes earlier than cytology (Avery, 2009 and Harini *et*

al.2022). Chemotherapy is the elected treatment to achieve remission and prolong survival time in dogs suffering from canine lymphoma. Combination protocols (such as CHOP) are more efficient than single-agent ones, with systemic multiagent chemotherapy being the standard treatment regimen. Novel molecular techniques such as the PARR assay are essential in creating treatment opportunities in cases where FNA and histopathology are insufficient to arrive at a definitive diagnosis, thus increasing survival rates.

Acknowledgement

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Fig 1. Emaciated Boxer dog on the day of presentation



Fig 2. ECG lead 2: absence of P wave with low-voltage QRS complex

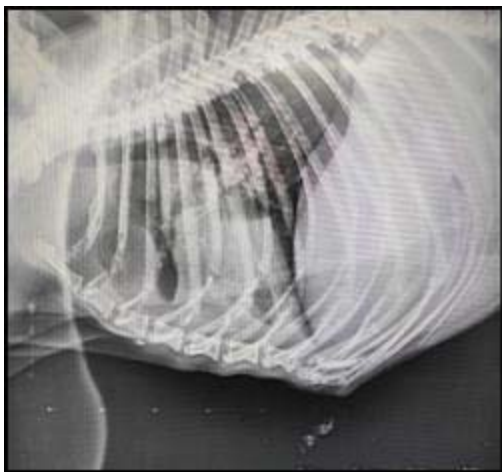


Fig 3: Thoracic Radiograph: Miliary pulmonary infiltration, a fluid shadow of the cranial lobe of the lung

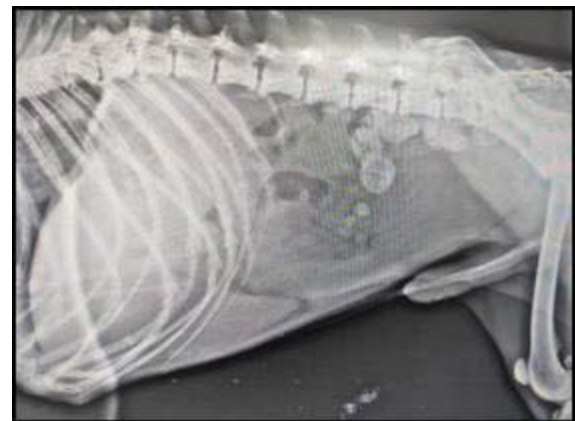


Fig 4: Abdominal Radiograph: Hepatomegaly, fluid shadow within the peritoneal cavity (ascites) and bowels obstructed with faeces



Fig 5: Abdominal ultrasonography: Splenomegaly

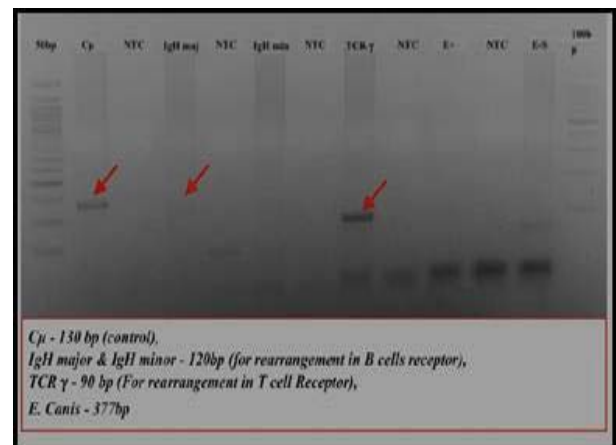


Fig 6: PARR Assay: clonal expansion in B cell receptor (BCR) and T cell receptor – γ (TCR- γ)